

WHAT IS CLAIMED IS:

1 Sub  
2 a<sup>1</sup> 7  
3  
1. An *in vitro* system capable of recapitulating regulated RNA turnover of an exogenously added preselected target RNA sequence comprising a cell extract and said target RNA sequence.

B 1 11  
B 2 2. The system of claim 1 wherein said regulated RNA turnover is selected from the group  
AB 3 consisting of AU-rich element regulated RNA turnover and C-rich element regulated RNA turnover.  
RNA deadenylation and degradation  
deadenylation and degradation  
deadenylation and degradation

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2 3. The system of claim 1 wherein said cell extract is isolated from lysed eukaryotic cells or tissues.

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a<sup>1</sup> 1  
2 Sub  
c3 } 4. The system of claim 1 wherein said cell extract is obtained from a cell line selected from the group consisting of HeLa cells and a T cell line.

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2 5. The system of claim 1 wherein said cell extract is prepared from cells comprising foreign nucleic acid.

a<sup>1</sup> 1 6. The system of claim 1 wherein said cell extract is prepared from cells which are infected,  
2 stably transfected, or transiently transfected. uninfected,

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a<sup>2</sup> 7. The system of claim 1 wherein said cell extract is partially purified.

8. The system of claim 1 wherein said cell extract is depleted of activity of proteins that bind polyadenylate. B

B 1 9. The system of claim 1 wherein said cell extract depleted of activity of proteins that bind  
2 polyadenylate is prepared by a method selected from the group consisting of:

3 (a) addition to said system of polyadenylate competitor RNA;

4 (b) sequestration of proteins that bind polyadenylate;

a<sup>5</sup> 5 (c) addition of a proteinase that inactivates a protein that binds to polyadenylate; and binds

6 (d) addition of an agent that prevents the interaction between polyadenylate and an  
a 7 endogenous macromolecule that binds to polyadenylate.

1 10. The system of claim 9 wherein said sequestration of proteins that bind polyadenylate  
a 2 is achieved by treatment of said extract with <sup>a</sup>an material that depletes  
3 macromolecules that bind polyadenylate selected from the group consisting of  
4 antibodies to proteins that bind polyadenylate, polyadenylate, and the combination  
5 thereof.

1 11. The system of claim <sup>9</sup>10 wherein said material is attached to a matrix.

a 1 12. The system of claim 1 wherein said target RNA sequence is selected from the group <sup>consisting</sup>  
2 of synthetic RNA, naturally occurring RNA, messenger RNA, chemically modified  
3 RNA, and RNA-DNA derivatives.

1 13. The system of claim 12 wherein said target RNA sequence comprises a 5' cap and a  
2 3' polyadenylate sequence.

1 14. The system of claim 1 wherein said target RNA sequence is selected from the group  
2 consisting of unlabeled target RNA sequence, labeled target RNA sequence, and the  
3 combination thereof.

1 15. The system of claim 14 wherein said labeled target RNA sequence is labeled with a  
2 moiety ~~is~~ selected from the group consisting of a fluorescent moiety, a visible  
3 moiety, a radioactive moiety, a ligand, and a combination of fluorescent and  
4 quenching moieties.

1 16. The system of claim 1 additionally comprising exogenously added nucleotide  
2 triphosphate.

1 17. The system of claim 16 wherein said nucleotide triphosphate is ATP.

1 18. The system of claim 1 further comprising a reaction enhancer.

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The system of claim 18 wherein said reaction enhancer is selected from the group consisting of polyvinyl alcohol, polyvinylpyrrolidone and dextran.

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The system of claim 19 wherein said reaction enhancer is polyvinyl alcohol.

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21.

A method for identifying an agent capable of modulating the stability of a target RNA sequence comprising

*in-vivo*

- (A) providing the system of claim 1;  
(B) introducing said agent into said system;  
(C) determining the extent of ~~turnover~~ *deadenylation and degradation* of said target RNA sequence; and  
(D) identifying an agent able to modulate the extent of said turnover as capable of modulating the stability of said target RNA sequence.

*Sub C7*

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The method of claim 21 wherein said system additionally comprises nucleotide triphosphate.

23.

The method of claim 22 wherein said nucleotide triphosphate is ATP.

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The method of claim 21 wherein said agent is an RNA stability modifying molecule.

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The method of claim 21 wherein said target RNA sequence is selected from the group consisting of unlabeled target RNA sequence, labeled target RNA sequence, and the combination thereof.

*Sub C9*

26.

The method of claim 25 wherein said labeled RNA sequence is labeled with a moiety ~~is~~ selected from the group consisting of a fluorescent moiety, a visible moiety, a radioactive moiety, a ligand, and a combination of fluorescent and quenching moieties.

27.

The method of claim 21 wherein said ~~monitoring~~ *determining* the extent of ~~turnover~~ *deadenylation and degradation* of said target RNA sequence comprises determining the extent of degradation of said labeled target RNA *sequence*.

1 28. The method of claim 21 wherein said modulating the stability of a target RNA  
2 sequence increases the stability of said target RNA sequence.

1 29. The method of claim 21 wherein said modulating the stability of a target RNA  
2 sequence decreases the stability of said RNA sequence.

1 <sup>23</sup> 30. The method of claim <sup>15</sup> 21 wherein said agent is capable of modulating the activity of a  
2 AU rich element binding protein or a C-rich element binding protein.

1 31. The method of claim 30 wherein said AU rich element binding protein is selected  
2 from the group consisting of a member of the ELAV protein family; AUF1;  
3 <sup>sup</sup> <sub>c10</sub> tristetrapolin; AUH; TIA; TIAR; glyceraldehyde-3-phosphate; hnRNP C; hnRNP  
4 A1; AU-A; and AU-B.

1 <sup>25</sup> 32. The method of claim <sup>24</sup> 31 wherein said member of the ELAV protein family is  
2 selected from the group consisting of HuR, Hel-N1, HuC and HuD.

1 33. A method for identifying an agent capable of modulating the <sup>in-vivo</sup> stability of a target  
2 RNA sequence in the presence of an exogenously added RNA stability modifier  
3 <sup>sup</sup> <sub>c11</sub> comprising

4 (a) providing the system of claim 1;

5 (b) introducing said RNA stability modifier into said system;

6 (c) introducing said agent into said system;

7 (d) determining the extent of <sup>deadenylation and degradation</sup> turnover of said target RNA sequence; and

8 (e) identifying an agent able to modulate the extent of said <sup>deadenylation and degradation</sup> turnover as capable  
9 of modulating the stability of said target RNA sequence in the presence of  
10 said exogenously added RNA stability modifier.

1 34. The method of claim 33 wherein said system additionally comprises nucleotide  
2 triphosphate.

1 <sup>sup</sup> <sub>c12</sub> 35. The method of claim 34 wherein said nucleotide triphosphate is ATP.

1 36. The method of claim 33 wherein said target RNA sequence is selected from the  
2 group consisting of unlabeled target RNA sequence, labeled target RNA sequence,  
3 and the combination thereof.

1 37. The method of claim 36 wherein said labeled RNA sequence is labeled with a moiety  
2 is selected from the group consisting of a fluorescent moiety, a visible moiety, a  
3 radioactive moiety, a ligand, and a combination of fluorescent and quenching  
4 moieties.

1 *Sub*  
2 *CG*  
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38. The method of claim 33 wherein said determining the extent of turnover of said target RNA sequence comprises determining the extent of degradation of said labeled target RNA.

1 39. The method of claim 33 wherein said RNA stability modifier increases the stability  
2 *sub* of said target RNA sequence.

1           40.     The method of claim 39 wherein said agent decreases the stability of said target RNA  
2           sequence increased by said RNA stability modifier.

41. The method of claim 33 wherein said RNA stability modifier decreases the stability of said target RNA sequence.

1 42. The method of claim 41 wherein said agent increases the stability of said target RNA  
2 sequence decreased by said RNA stability modifier.

1                    <sup>35</sup>  
                      ~~43~~.                    <sup>26</sup>                    ~~37~~                    The method of claim ~~37~~ wherein said agent is capable of modulating the activity of a  
2                    AU rich element binding protein or a C-rich element binding protein.

1 44. The method of claim 43 wherein said AU rich element binding protein is selected  
2 sub from the group consisting of a member of the ELAV protein family; AUF1;  
3 C15 tristetrapolin; AUH; TIA; TIAR; glyceraldehyde-3-phosphate; hnRNP C; hnRNP  
4 A1; AU-A; and AU-B.

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The method of claim 44 wherein said member of the ELAV protein family is selected from the group consisting of HuR, Hel-N1, HuC and HuD.

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46.

A method for identifying an agent capable of modulating the deadenylation of a target RNA sequence comprising

- (A) providing the system of claim 1 in the absence of a nucleotide triphosphate;
- (B) introducing said agent into said system;
- (C) monitoring the deadenylation of said target RNA sequence in said system; and
- (D) identifying an agent able to modulate the extent of said deadenylation as capable of modulating the deadenylation of said target RNA sequence.

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47.

A method for identifying an agent capable of modulating <sup>regulated</sup> the deadenylation and degradation of a target RNA sequence comprising

- (A) providing the system of claim 1 in the presence of a nucleotide triphosphate;
- (B) introducing said agent into said system;
- (C) monitoring the deadenylation and degradation of said target RNA sequence in said system; and
- (D) identifying an agent able to modulate the extent of said deadenylation and degradation as capable of modulating the deadenylation and degradation of said target RNA sequence.

48.

A method for identifying an agent capable of modulating cell growth or cell differentiation in a mammal comprising determining the ability of said agent to modulate the stability of a target RNA sequence involved in the modulation of cell growth or differentiation in accordance with claim <sup>21</sup>~~19~~.

49.

The method of claim 48 wherein said agent capable of modulating cell growth or cell differentiation intervenes in cellular transformation.

1 50. The method of claim 48 wherein said agent ~~capable of modulating cell growth or cell~~  
2 differentiation intervenes in immune dysregulation.

1 51. A method for identifying, characterizing or isolating an endogenous molecule  
2 suspected of participating in the deadenylation or degradation of RNA or regulation  
3 thereof comprising  
4 (A) providing the system of claim 1;  
5 (B) introducing said ~~protein~~ <sup>endogenous molecule</sup> suspected of participating in the regulation of  
6 RNA ~~turnover~~ <sup>deadenylation and degradation</sup> into said system;  
7 (C) monitoring the stability of said target RNA sequence in said system; and  
8 (D) identifying, characterizing or isolating said endogenous molecule able to  
9 modulate said deadenylation or degradation as capable of participating in  
10 the deadenylation or degradation of RNA or regulation thereof.

1 40 52. The method of claim ~~51~~ <sup>39</sup> wherein said molecule suspected of participating in the  
2 deadenylation or degradation of RNA or regulation thereof is protein or RNA.

1 53. A kit for monitoring the stability of a preselected target RNA sequence under  
2 conditions capable of recapitulating regulated RNA turnover, said kit comprising:  
3 (a) cell extract <sup>supernatant</sup> depleted of activity of proteins that bind polyadenylate;  
4 (b) other reagents; and  
5 (c) directions for use of said kit.

1 45 54. The kit of claim ~~53~~ <sup>44</sup> further comprising nucleotide triphosphates, a reaction enhancer,  
2 a target RNA sequence, or any combination thereof.

1 55. A method for identifying an agent capable of modulating the <sup>of</sup> degradation <sup>of</sup> a target  
2 RNA sequence in the absence of deadenylation comprising  
3 (A) providing a cell extract in the presence of a nucleotide triphosphate;  
4 (B) introducing said agent into said cell extract; and  
5 (C) monitoring the degradation of said target RNA sequence in said extract.